

The University of Chicago

THE FLUORESCENCE OF CHLOROPHYLL AND PHOTOSYNTHESIS

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE
PHYSICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMISTRY

1940

By

THEODORE THOMAS PUCK

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T. T. PUCK¹

I. INTRODUCTION

The fluorescence of chlorophyll in photosynthesizing plants has been the subject of a number of recent investigations. Such studies should throw light on the mechanism of photosynthesis, for, even though the amount of energy involved in the fluorescence is too small to affect the energy balance of the chemical reactions, the changes in the intensity of fluorescence of the chlorophyll indicate changes in the concentration of molecules (intermediates of photosynthesis) capable of taking up its excitation energy and thereby quenching the fluorescence.

Kautsky and his coworkers (7), Franck and Wood (5), Ornstein *et al.* (11, 18, 19, 20), and McAlister and Myers (9)² have shown that a characteristic series of changes in the fluorescence intensity of the chlorophyll in plants occurs at the beginning of an illumination, during the induction period of photosynthesis. This paper presents further observations both on the variations in fluorescence during the induction period and on the constant fluorescence which obtains during the steady state of photosynthesis. An interpretation of these phenomena is presented, based in part on the theoretical mechanism for photosynthesis due to Franck and Herzfeld (4).

II. APPARATUS

Our fluorescence-measuring apparatus is diagrammed in figure 1. Light from a 500-watt tungsten projection lamp, equipped with a transformer voltage regulator, was focussed by condenser lenses to form a uniformly illuminated spot 1 in. in diameter on the leaf or the algae. The leaf was firmly supported in a closed brass chamber equipped with a front glass window. The temperature of the leaf was controlled by circulating water through a copper coil soldered to the outside of the back wall of the chamber against which the leaf was pressed. The chamber was provided with gas inlet and outlet tubes, so that various gas mixtures could be used. All the gases were first saturated with water before being admitted to the leaf. To study the fluorescence of algae with the same apparatus, a glass tube containing a water suspension of the algae was placed at the focus of the exciting light.

¹ Gustavus F. Swift Fellow in Chemistry.

² The experiments of McAlister and Myers were performed simultaneously with ours. The data presented in these two papers supplement each other in many ways.

The exciting light was restricted to wave lengths between 4000 and 6000 Å. by a filter system consisting of a Corning infrared filter, 1 cm. of a quinine sulfate solution, and 5 cm. each of a saturated ferrous ammonium sulfate solution and a 7 per cent cupric sulfate solution.

The fluorescent light from the leaf was focussed by a lens onto a cesium photocell (R. C. A. 922) before which was placed a red glass filter (Corning #241; cut-off near 6300 Å.), so that the main wave lengths of the chlorophyll fluorescence were transmitted, but none of the exciting light entered the photocell. The efficiency of the light-filtering system was demonstrated by the absence of any appreciable response of the galvanometer when a piece of white paper was placed in the leaf holder to act as a reflecting surface.

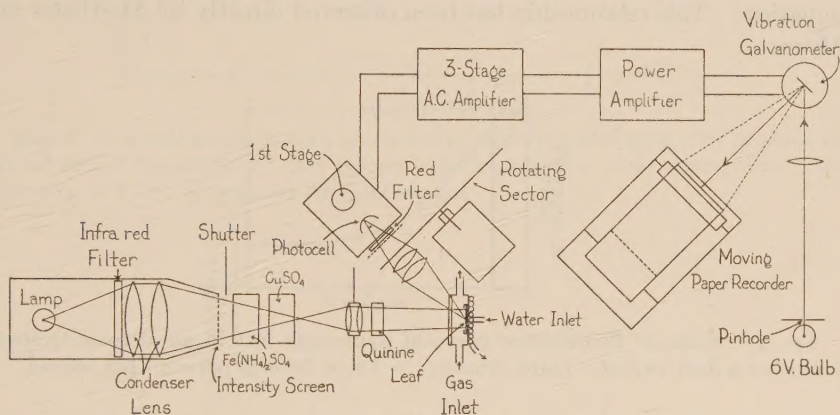


FIG. 1. Diagram of the apparatus used in recording the time changes of intensity of the fluorescent light from leaves illuminated with blue light.

A rotating sector placed just before the photocell lens interrupted the light beam two hundred and forty times per second. The resulting photocell current was amplified by a five-stage A.C. amplifier. The output of the amplifier was led to a vibration galvanometer, the mirror of which made a complete excursion for each pulse of light on the photocell. The oscillations of the galvanometer, proportional in amplitude to the intensity of fluorescence, were recorded directly on moving photographic paper. The speed of the recording machine in which the photographic roll was supported could be varied over wide limits. A sample fluorescence recording produced by this apparatus is shown in figure 15.

The range of light intensities used varied from about 0.2×10^4 to 20.0×10^4 ergs per cm^2 per second. The lower light intensities were produced by the insertion of calibrated metal screens in the light path. Light intensities were measured with a Weston photronic cell which had been calibrated in energy units for the particular wave-length region used by

means of a thermopile and a standard lamp from the United States Bureau of Standards.

Figure 2 presents a typical curve showing the variation of fluorescence intensity with the time of illumination in a normal hydrangea leaf. Most of our experiments were made with this plant because its stomata are reputed to be immobile. We have obtained fundamentally the same fluorescence curve in many different higher plants.

The experiments of Blinks and Skow (2) on the rate of oxygen liberation during the first minute of illumination of a plant previously kept in the dark have shown that the reaction responsible for the sudden outburst in fluorescence is connected with a temporary hindrance in the oxygen evolution. As a result, there exists a direct antiparallelism between the rate of photosynthesis and the fluorescence intensity at the beginning of illumination. This relationship has been observed directly by McAlister and Myers.

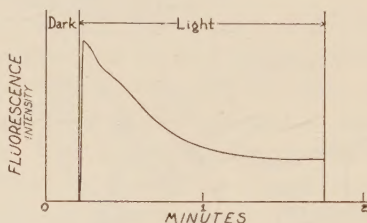


FIG. 2. "Normal" fluorescence curve of hydrangea leaf in air at room temperature after a dark period. Light intensity = 8.8×10^4 ergs per cm.² per second.

III. EXPERIMENTS

A. Fluorescence during the induction period of photosynthesis

The fluorescence-time curve of a photosynthesizing leaf (figure 2) has three distinct phases, two of which occur during the induction period of photosynthesis. The first is a very rapid rise in fluorescence, and it is followed by a second phase wherein the fluorescence intensity slowly falls. In the third phase, which corresponds in time to the steady state of photosynthesis, the fluorescence intensity remains constant.

(1) The initial rise

Figure 3 presents a typical high-speed fluorescence record. The initial fluorescence intensity at the start of an illumination is practically the same as the steady-state value obtained after several minutes. Within a fraction of a second after the leaf is exposed to the light, however, the fluorescence begins to rise rapidly and reaches a maximum in about 1 sec. The magnitude of this fluorescence outburst depends upon the intensity of the exciting

light. It approaches zero with zero light intensity, but above a certain critical light intensity further increase in the exciting light produces only a small increase in the fluorescence outburst, as shown in figure 4.

The velocity of the reaction producing the outburst also increases with the intensity of the incident light, though here too the curve (showing the reciprocal of the time necessary to reach one half of the maximum intensity, as a function of the light intensity) eventually bends toward the time axis (see figure 5). Plotted in the same figure are the values of $1/t$ obtained

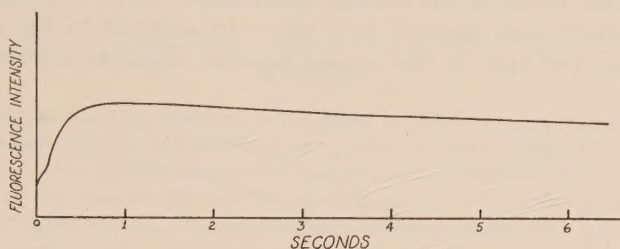


FIG. 3. A record made at high speed with a rapidly opening shutter to show the initial rise of fluorescence. One per cent carbon dioxide; room temperature; light intensity = 2.7×10^4 ergs per cm^2 per second.

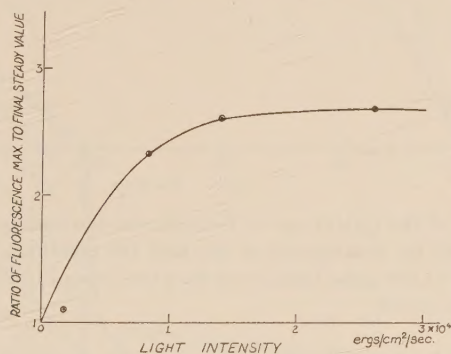


FIG. 4. As the intensity of the light is increased, the peak value of the fluorescence outburst increases up to a limiting value roughly three times the intensity of the final steady state.

at a temperature of 0°C . It is obvious that the rate of the first rise in fluorescence is practically the same at 23°C . and at 0°C . for any light intensity.³

³ That $1/t$ is a little smaller at 0°C . than at 23°C . is a secondary effect due to the fact that, although the rate of rise in fluorescence is the same at both temperatures, the subsequent drop in fluorescence intensity is very much retarded at 0°C . As a result, therefore, at 0°C . there is less overlapping of the fluorescence fall on the fluorescence rise, so that the maximum is reached somewhat later.

Both the rate and the extent of this first reaction are independent of the concentration of carbon dioxide. The height of the fluorescence maximum and the time necessary to attain it remain constant, regardless of whether the atmosphere surrounding the leaf be carbon dioxide-free air, normal air, or air containing admixtures of 1, 4, or 20 per cent of carbon dioxide, provided the previous treatment of the leaf was the same in every case.

(2) The decay of fluorescence

The second phase of the normal fluorescence curve is a slow decay in intensity which lasts about 2 or 3 min. In contrast to the first rise in fluorescence, the rate of this second reaction depends markedly on the

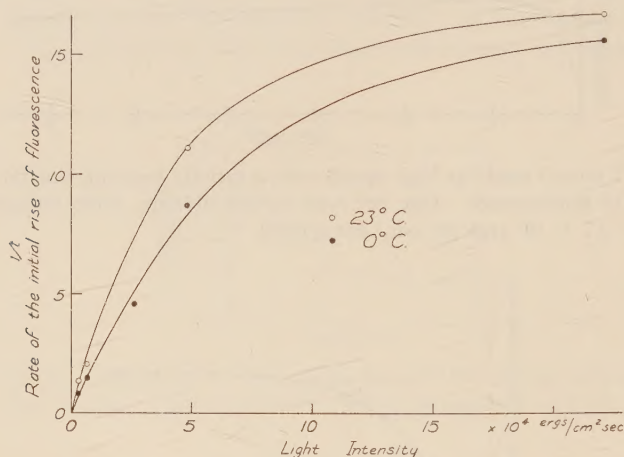


FIG. 5. The rate of the initial rise of fluorescence (as measured by the reciprocal of the time necessary for attainment of one-half the maximum intensity) increases with light intensity in the same fashion as does the rate of photosynthesis (compare with figure 17, lower curve).

concentration of carbon dioxide. The experiments recorded in figure 6 indicate how the rate of fluorescence decay is retarded when the concentration of carbon dioxide in the leaf is increased.

The effect of carbon dioxide in retarding the rate of the fluorescence decay is strikingly exhibited in the curve of figure 7, which records an experiment wherein 20 per cent carbon dioxide was rapidly introduced and removed again at various points during the induction period. This effect is so marked that it can be used to trace the flow of carbon dioxide diffusing through a leaf.

In an atmosphere of 1 per cent or slightly more of carbon dioxide, however, somewhat different curves are often obtained, exhibiting additional fluorescence maxima after about the first 2 to 3 min. of illumination

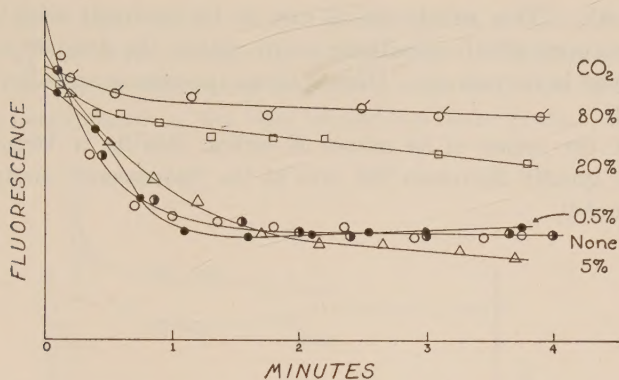


FIG. 6. The decay of fluorescence is inhibited by high concentrations of carbon dioxide. A single leaf was exposed for 4 min. with 1 hr. dark recovery between exposures, as follows: No carbon dioxide; 0.5 per cent carbon dioxide; 5 per cent carbon dioxide; 20 per cent carbon dioxide; 80 per cent carbon dioxide; no carbon dioxide repeated (half-filled circles). These measurements were made with a visual photometer, using as the exciting light the lines 546, 492, and 436 $m\mu$ from a mercury lamp. Total intensity on the leaf was 1.7×10^4 ergs per $cm.^2$ per second.

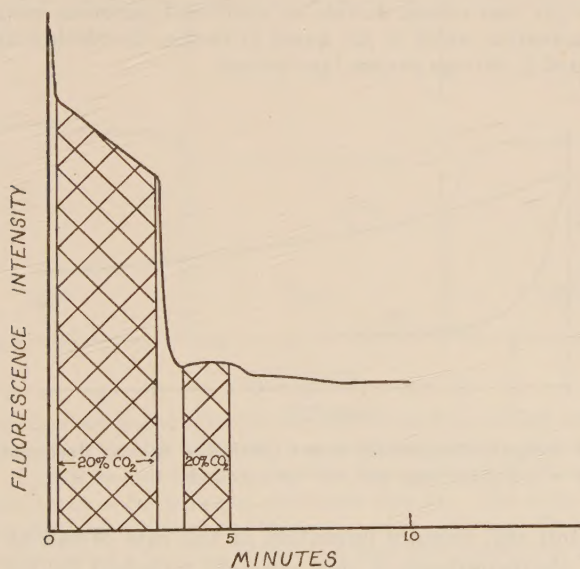


FIG. 7. A leaf exposed to light in the absence of carbon dioxide suddenly had 20 per cent carbon dioxide passed into the chamber 0.2 min. after the beginning of illumination. The rate of decay is immediately retarded, showing that the carbon dioxide penetrates the leaf and has its effect within a few seconds. In about 10 sec. after the carbon dioxide is removed, the fluorescence again starts to decrease rapidly. Light intensity = 8.8×10^4 ergs per $cm.^2$ per second.

(see figure 8). (This maximum is not to be confused with the small secondary maxima which sometimes occur within the first 20 sec. of illumination, even in normal air. One of these appears as an inflection point in figure 8.)

Similar to the action of an excess of carbon dioxide, a decrease in the temperature greatly decreases the rate of the fluorescence decay, as indicated in figure 9.

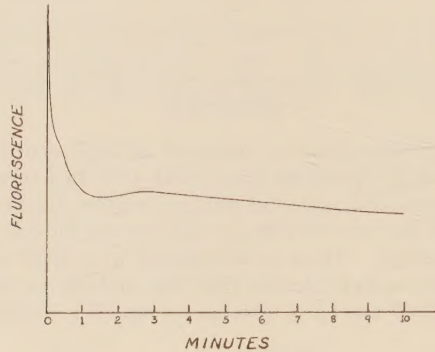


FIG. 8. In 1 per cent carbon dioxide an additional maximum occurs at about 3 min. after illumination which is not found in carbon dioxide-free air. The light intensity was 0.82×10^4 ergs per cm^2 per second.

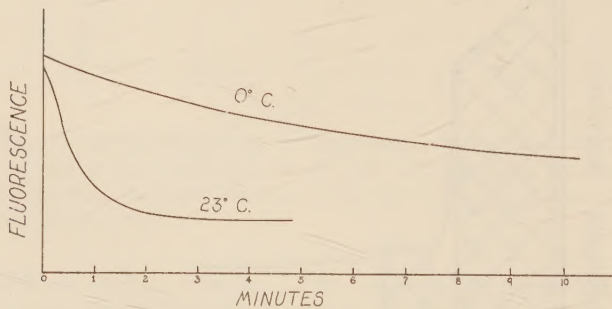


FIG. 9. Low temperature greatly slows down the rate of decay of fluorescence. Light intensity = 4.4×10^4 ergs per cm^2 per second.

A smaller but still definite inhibition of the rate of fall of fluorescence occurs when photosynthesis is very strongly poisoned by the addition of 2 per cent of gaseous hydrogen cyanide, as the curve of figure 10 shows.

The fluorescence decay indicates a removal of the substance whose formation during the first second of illumination is marked by the sharp rise of fluorescence intensity. This substance is removed in the dark as well as in the light, as the following series of experiments show: A leaf was

illuminated for 3 sec., given a dark period, and then re-illuminated as in figure 11. If the dark period after a 3-sec. flash of light is greater than ~ 20 sec., the highly fluorescing material produced by the first flash has completely disappeared by the time the second flash occurs. In this case,

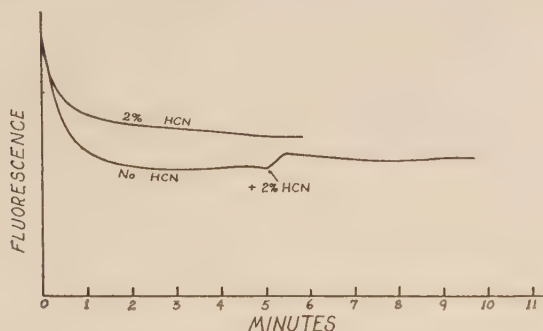


FIG. 10. Addition of 2 per cent of hydrogen cyanide to a gas mixture of air, plus 1 per cent of carbon dioxide, slows down the rate of fluorescence decay. Light intensity = 0.5×10^4 ergs per cm^2 per second.

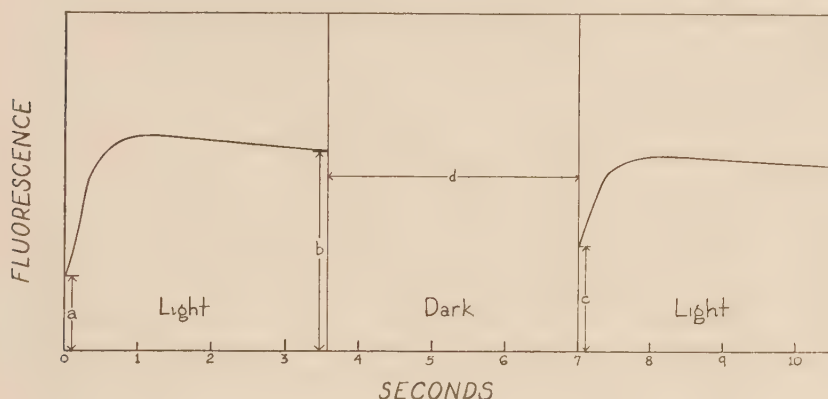


FIG. 11. A high-speed record of the initial stage of the fluorescence curves. A short exposure, then a dark period followed by a second exposure, were made as illustrated above. It will be noticed that after the first exposure the fluorescence starts at a higher value (c) on re-illumination after dark time (d). The drop of fluorescence ability in short dark periods from the final value of one curve to the initial value of the next was measured as a function of the dark time from curves like these and summarized in figure 12. The data are shown in table 1.

the two fluorescence curves so obtained are superimposable. But if the dark period between the light flashes is smaller than 20 sec., some of this substance still remains at the end of the dark interval. In this case, the fluorescence at the starting point is higher, and the maximum height

achieved during the first second of re-illumination is lower. The data are given in table 1.

TABLE 1
Disappearance in the dark of the highly fluorescing material produced in the light
(figures 11 and 12)

(1)	2	(3)	(4)	(5)	(6)	(7)
DARK TIME BETWEEN LIGHT FLASHES <i>d</i>	FINAL FLUORESCENCE INTENSITY OF PREVIOUS ILLUMINATION <i>b</i>	DIFFERENCE BETWEEN THE INTENSITY AT THE MOMENT OF INTERRUPTION OF ILLUMINATION AND THE INITIAL VALUE <i>b - a</i>	INITIAL INTENSITY AFTER THE DARK TIME <i>c</i>	LOSS OF FLUORESCENCE ABILITY IN THE DARK TIME <i>b - c</i>	PER CENT LOSS OF FLUORESCENCE ABILITY IN THE DARK TIME $\frac{100 (b - c)}{c}$	PER CENT OF FLUORESCENCE ABILITY REMAINING AFTER DARK TIME 100 - VALUE IN COLUMN 6
<i>seconds</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	<i>per cent</i>	<i>per cent</i>
0					0	100
0.8	56	27	44	12	30	70
1.5	73	44	51	22	43	57
1.8	50	21	31	19	61	49
2.3	77	48	45	32	71	29
2.8	81	52	42	39	93	7
3.0	54	25	33	21	64	36
9.2	65	36	33	32	97	3
∞		(<i>a</i> = 29)			100	0

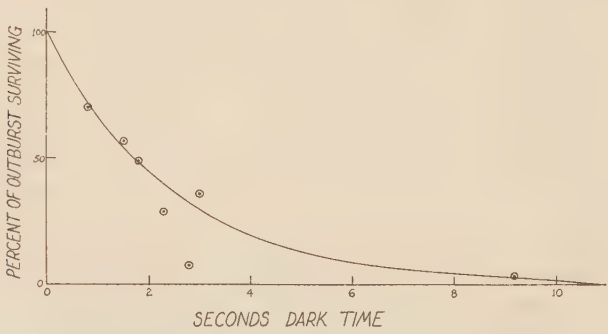


FIG. 12. The survival of the high fluorescence-promoting substances after illumination ceases. Measured by the starting height of curves made after 3-sec. illumination, followed by dark periods of various durations. One such curve is shown in figure 11. The data are presented in table 1. The curve corresponds to the equation $y = e^{-0.406t}$. The half-life is 1.7 sec.

Figure 12 shows the percentage loss of the fluorescent material as a function of the dark periods. The half-life, under the conditions of the experiment, is about 1.7 sec.

The lifetime of the substance responsible for the increased fluorescence yield was also studied in another way. A leaf was illuminated with a short intense light flash, which was followed by an indicator light beam of very weak intensity. The weak light is incapable of producing an appreciable amount of photochemical reaction, but merely allows one to follow the history of the fluorescing substances previously produced. In figure 13 are shown the results of illuminating a leaf with a bright flash of 0.07 min. duration, followed by a sudden transition to weaker light. It can be seen that the fluorescence during the first second or two of the weak illumination is higher than normal. However, if the bright exposure is prolonged, as

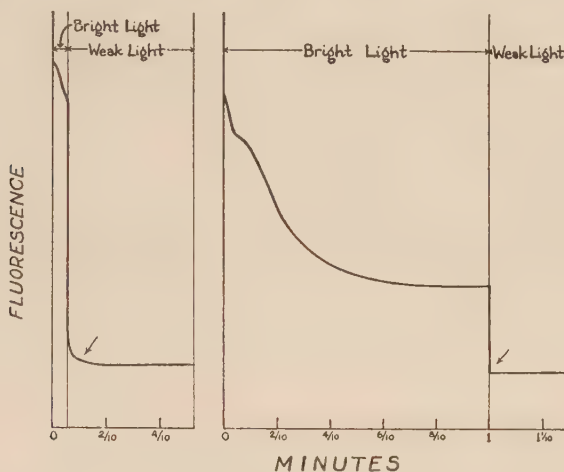


FIG. 13.⁴ After a flash of bright light the fluorescence of a leaf in weak light remains high for a few seconds until the substance responsible for the outburst of fluorescence is removed by a thermal reaction. After a long period of bright light, however, there is none of that substance present, as is indicated in the second half of the figure. The intensities used were as follows: bright light = 2.4×10^4 , weak light = 0.18×10^4 ergs per cm.² per second. A screen in front of the photocell was raised by a solenoid at the same time that an electrically operated shutter cut off the bright light.

in the second part of the figure, there is no indication whatever of the survival of the highly fluorescing material when the light intensity is reduced, as shown in table 2.

When the temperature of the leaf was lowered to 0°C., the time over which survival of these highly fluorescing materials was observable increased. Thus, after a 6-sec. illumination with a light intensity of 4.8

⁴ In figure 13, as in all subsequent figures, whenever the intensity of the exciting light was suddenly changed, filters were simultaneously inserted or removed from before the photocell so that the deflection of the galvanometer always remained conveniently measurable. Correspondingly, therefore, the size of the units in which the fluorescence intensity is expressed also changes.

$\times 10^4$ ergs per cm^2 per second, reduction of the light intensity to 0.6×10^4 ergs per cm^2 per second at room temperature resulted in a survival of the strongly fluorescing material for 2 sec. The same experiment performed at 0°C . resulted in material which survived for about 5 sec. at the lower light intensity. Since the gradual disappearance of this fluorescing material is superimposed on a normal falling fluorescence curve, it is difficult to determine precisely when the highly fluorescing material has completely disappeared. The numbers presented, therefore, are to be regarded as qualitative only.

If a leaf is illuminated with light of moderate intensity and then, after steady-state conditions have been reached, the light intensity is suddenly increased, a new outburst of fluorescence is obtained, as is shown in figure 14a, but if the light intensity first used is high enough to produce light saturation of photosynthesis, further increase in its intensity produces no new fluorescence outburst. Thus (figure 14b) no new fluorescence maxi-

TABLE 2*

DURATION OF FLASH OF BRIGHT LIGHT (2.6×10^4 ERGS PER CM^2 PER SECOND)	TIME OF SURVIVAL OF HIGHLY FLUORESCING MATERIAL IN WEAK LIGHT ($0.13 \times$ 10^4 ERGS PER CM^2 PER SECOND)
<i>seconds</i>	<i>seconds</i>
0.6	3.3
1.2	3.8
3	2.0
6	1.9
12	0
24	0
60	0

* See figure 13.

imum is obtained if a leaf, already illuminated with a light intensity of 7.4×10^4 ergs per cm^2 per second for several minutes, is subjected to an additional illumination of 4.8×10^4 ergs per cm^2 per second, but a definite outburst is obtained if the original intensity was only 0.5×10^4 ergs per cm^2 per second (figure 14a).

The value 7.4×10^4 ergs per cm^2 per second is just about that necessary to produce photosynthesis saturation in these leaves (figure 17).

Exactly the same relations were found by McAlister (8) to hold for the induction loss of photosynthesis; i.e., if the light intensity was increased from an initially low value, a new induction period of photosynthesis occurred, but if the original exciting light had been high, no new induction period was obtained.

Experiments were also performed with *Chlorella* and *Scenedesmus*. Here it was found possible to obtain two different types of fluorescence curves depending on the previous treatment of the plants. Fresh cultures

gave curves exactly like those of the hydrangea leaves. Old cultures, however, gave curves like that reproduced in figure 15. This type of fluorescence curve is exactly like that given by the algae of Wassink and Katz (19).

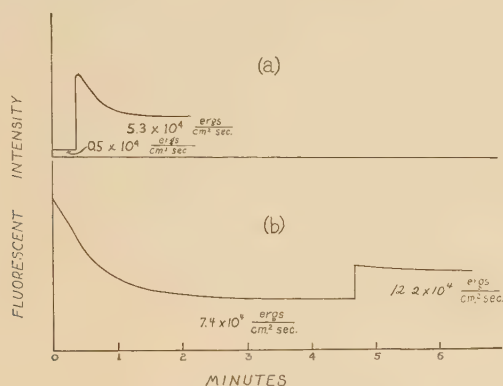


FIG. 14. (a) When a leaf which has been illuminated at low intensity is suddenly given brighter light, a fluorescence outburst occurs and decays again just as if the leaf had been in the dark before the bright exposure. (b) As compared with (a), however, a leaf exposed first to high intensity and then to a still higher one gives no outburst if the first intensity is above that required to produce light saturation of photosynthesis.

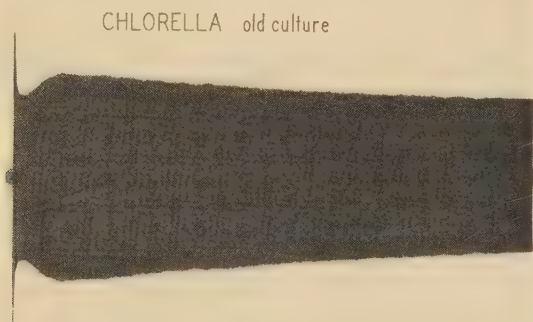


FIG. 15. Fluorescence curve of the alga *Chlorella* (old culture)

B. Fluorescence during the steady state of photosynthesis

(1) Fluorescence of leaves under standard conditions

The steady-state fluorescence was first studied by Wassink, Vermeulen, Reman, and Katz (20), who, working on the alga *Chlorella*, reported the

fluorescence intensity to be directly proportional to the intensity of the exciting light even well into the region of saturation. McAlister and Myers (9) found an increase in the fluorescence yield of young wheat plants when the amount of carbon dioxide available was deficient so that photosynthesis was light-saturated, but were unable to carry out experiments at light intensities high enough to attain saturation in the presence of an adequate supply of carbon dioxide. Our experiments were performed with hydrangea leaves in the presence of 1 per cent of carbon dioxide. We have found the fluorescence yield to be constant only at low light intensities where the rate of photosynthesis is proportional to the intensity of the exciting light. In the region of light saturation, however, the quantum yield of fluorescence increases.

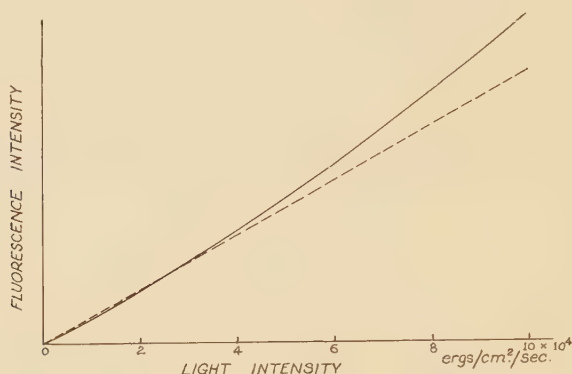


FIG. 16. Steady-state fluorescence intensity as a function of the exciting light, showing that the fluorescence increases more than proportionally to the incident illumination.

One of our sample experimental curves is presented in figure 16. In order to extend the range over which fluorescence measurements could be performed without fear of distortions introduced by non-linearity in the amplifying system, however, another method was adopted wherein a fluorescence standard consisting of a solution of chlorophyll in alcohol was used as a control. That the fluorescence of this alcoholic solution was strictly proportional to the intensity of the exciting light throughout the range used in these experiments was established by a light summation method.

The light intensity was varied by the insertion of neutral filters into the path of the exciting beam. It was insured that the steady-state fluorescence had been reached in every case by accepting readings only after they had remained constant for at least 1 to 2 min.

Our experimental results are summarized in figure 17. The quantity y is the yield of fluorescence at any value of the intensity of the exciting light,

and y_0 is the constant fluorescence yield which obtains at very low light intensities. In the same figure is presented a curve showing the rate of photosynthesis as a function of the light intensity in a hydrangea leaf.

An increase in the quantum yield of fluorescence with the light intensity was also observed when the atmosphere surrounding the leaf consisted of normal air alone or of a mixture of 97 per cent nitrogen, 1 per cent carbon dioxide, and 2 per cent oxygen.

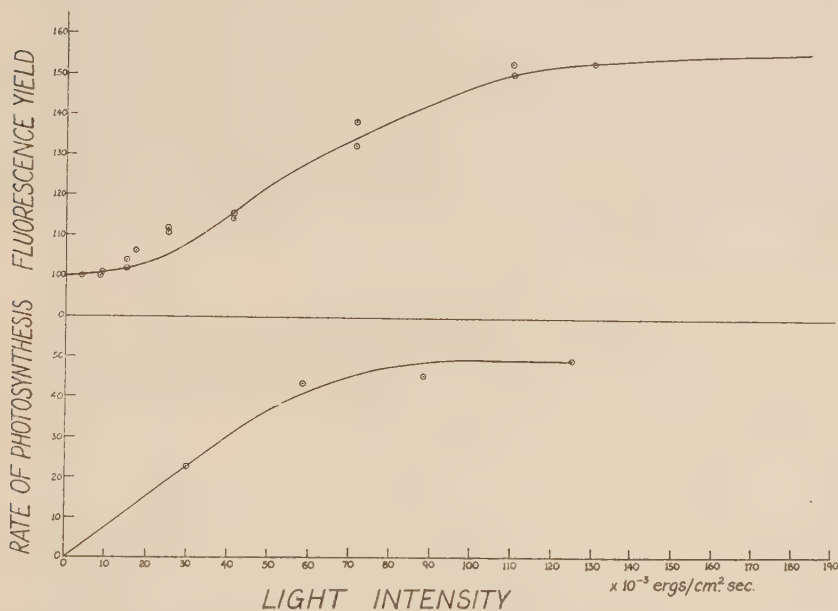


FIG. 17. Upper: Relative fluorescence yield; y_0 is the quantum yield of fluorescence at low light intensities; y is the yield at any light intensity. Gas phase is 1 per cent carbon dioxide in air. The different kinds of points represent separate experiments on different leaves. A certain amount of variation from leaf to leaf is unavoidable because of the differences in the internal conditions of various leaves. Lower: Rate of photosynthesis in an atmosphere of 1 per cent carbon dioxide, as measured by the number of cubic millimeters of oxygen liberated per minute in a Warburg manometer.

That this rise in fluorescence intensity is not an effect of excessively strong irradiation alone, but is directly related to the photosynthetic processes, was shown by the following experiment: A leaf was illuminated for 5 to 6 min. with a light intensity of about 1.7×10^4 ergs per cm^2 per second, first in an atmosphere of 5 per cent carbon dioxide, then, after a 30-min. dark recovery period, in air from which most of the carbon dioxide had been removed by bubbling through a solution of sodium hydroxide. As a check the illumination in 5 per cent carbon dioxide was repeated after another dark period. The weak light intensity used in this experiment

was sufficient to attain light saturation (or at least come very close to it) in the case where the concentration of carbon dioxide was extremely limiting, but not in the case where 5 per cent of carbon dioxide was present. The value of the steady fluorescence intensity in the carbon dioxide-free air was about 19 per cent higher than in the air containing 5 per cent of carbon dioxide (see table 3).

In the course of the measurements on the fluorescence yield it was found that, at very high illuminations only, a steady-state fluorescence intensity cannot always be obtained. Instead the fluorescence may continue to fall at a very slow rate—about 0.5 to 2 per cent per minute—even after 30 min. of illumination. Whether a constant fluorescence is attained in strong light seems to depend on variable internal factors within the leaf. For example, with an illumination of 13.0×10^4 ergs per cm.² per second in an atmosphere of 1 per cent carbon dioxide in air, the fluorescence of one leaf was constant after 3 min., whereas that of another was still falling after 10 min. of illumination.

TABLE 3
Steady-state fluorescence intensity

5 PER CENT CARBON DIOXIDE	CARBON DIOXIDE-FREE AIR	5 PER CENT CARBON DIOXIDE
	38	31
33	36	31
33	38	32

This failure to achieve true constancy at high light intensities is probably due to photooxidation processes.⁵ That this slow decay in fluorescence intensity is an extraneous effect superimposed upon the normal fall in fluorescence occurring in the first minute or two of illumination is shown by the following fact: If, after a constant fluorescence at a low light intensity is achieved, the illumination is increased to a high value and kept at this point for several minutes so that the slow decay has set in, and is then reduced again to the original low intensity, the final steady fluorescence is weaker than that obtained at first. If the light intensity is never made too great, however, the fluorescence at each value of the exciting light is constant and reproducible. In the foregoing experiments, therefore, if it was found that a given leaf did not soon achieve constancy at a high light intensity, the reading for that intensity was disregarded and only those for intensities low enough to give satisfactorily constant fluorescence were accepted.

McAlister and Myers, working with wheat plants, also found an increas-

⁵ Cf. Myers and Burr (10) and also results (to be published soon) by Franck and French (3) on photooxidation.

ing fluorescence yield in the region where the rate of photosynthesis becomes independent of the light intensity, but only when such light saturation was due to deficiency in the carbon dioxide concentration. They were unable to reach light saturation in the presence of a sufficient concentration of carbon dioxide, so that the behavior of wheat under these conditions is unknown. The difference which these authors observed in fluorescence curves taken in the absence of oxygen and in its presence is, in our opinion, a secondary effect, due to the fact that saturation of photosynthesis occurs at a lower light intensity in oxygen.

The difference in behavior of the *Chlorella* studied by Wassink, Vermeulen, Reman, and Katz (20) is probably one of degree only, since in the published curves the one or two points measured at the highest light intensities used systematically fall above the extrapolated straight line. The difference between the fluorescence of these plants and of leaves probably lies only in the fact that the rise in leaves starts before normal saturation is reached, whereas in these algae the increase in fluorescence yield occurs in a region beyond light saturation.

We next investigated whether the highly fluorescing substance responsible for the increase in the steady-state fluorescence at high light intensities has a lifetime similar to that of the material which produces the increase in fluorescence during the induction period. Using a method which would have revealed the existence of a very short lifetime, we found no indication of the survival of the highly fluorescing substances for a period of from 0.1 to 2 sec. after the light intensity was reduced. In the arrangement used a leaf was illuminated for 5 min. by two light sources, before one of which was placed a photographic shutter. When the shutter was closed, the light intensity striking the leaf dropped from 1.1×10^5 to 0.26×10^5 ergs per cm.² per second. The recorder was operated at the highest speed available.

Under these conditions the record could not be read for about the first 0.1 sec., because the galvanometer required two or three vibrations to adjust itself to so great a change in deflection. In the remainder of the record, however, it was impossible to detect any rapid decline of the fluorescence which could be interpreted as a short survival of strongly fluorescing material present at high illumination. The upper limit for such a lifetime may therefore be placed at 0.1 sec. From these observations it follows that the chemical process responsible for the outburst of fluorescence at the beginning of an illumination period is a different one from that which causes the rise of the steady fluorescence at high light intensities.

Our measurements had not excluded the possibility that the lifetime of the highly fluorescing material of the steady state was in reality very long, —30 sec. or a minute. In order to test this possibility, these experiments

were repeated with the recorder running at a much lower speed. Here we found that, on going from a very strong illumination to a very weak one, an effect opposite to that expected was obtained. The fluorescence intensity first drops to a point lower than its equilibrium value and then gradually recovers, reaching a constant level in 0.5 to 3 min.

This phenomenon (figure 18) was not observed unless the difference between the upper and the lower intensities used was very large. Thus, when the decrease in light was only from 13×10^4 to 1.8×10^4 ergs per cm^2 per second, either no rise whatever in the fluorescence intensity or else a very small one was observed. But when the light intensity was decreased from 13×10^4 to 0.81×10^4 ergs per cm^2 per second, a rise of 17 per cent was observed, and when the upper and lower light intensities were 6×10^4 and 0.37×10^4 ergs per cm^2 per second, respectively, increases in fluorescence intensity as high as 40 to 50 per cent were often obtained.⁶

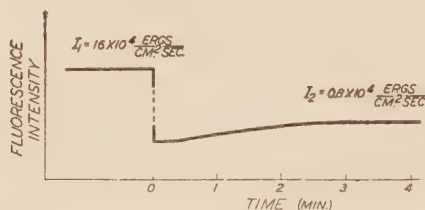


FIG. 18. Sudden lowering of the intensity of the exciting light after the steady state has been reached causes the fluorescence intensity to fall to a point lower than its equilibrium value, after which the fluorescence slowly rises.

The magnitude of the rise obtained varied from leaf to leaf, even though all external experimental conditions were kept constant. With a given leaf, however, a larger effect is obtained with an atmosphere of normal air than with air enriched by the addition of .1 per cent of carbon dioxide. The occurrence of this effect makes it impossible to determine by such measurements the lifetime of the material with the higher fluorescence yield which is produced by the strong illumination. It may well be that the effect just described compensates and often overcompensates the fall of the fluorescence which would otherwise occur because of the disappearance of the highly fluorescing material in the weak light.

⁶ This overshooting and recovery phenomenon in no way interfered with the experiments testing the constancy of the fluorescence yield with light intensity. In most of those experiments the decrease in light intensity between consecutive measurements was too small to produce any such effect. However, when experiments were performed with changes in light intensity great enough to cause this overshooting, the final equilibrium value was the one accepted. Both types of experiments gave the same results.

(2) Fluorescence in leaves the photosynthesis of which is inhibited

(a) *Inhibition by low temperature:* The steady-state fluorescence was also studied under conditions where photosynthesis was inhibited by low temperature and by gaseous hydrogen cyanide and by an excess of carbon dioxide. Leaves were illuminated under normal conditions for 5 or 10 min., so that the induction period had been completed. Then, for the low-temperature experiments, the leaf chamber was suddenly cooled to 0°C. by circulating ice water at a rapid rate through a copper coil attached to the back of the metal leaf holder. Similarly, where the effect of hydrogen cyanide in the steady state was to be studied, the composition of the gas mixture passing over the leaf was changed from air containing 1 per cent of carbon dioxide to air containing 1 per cent of carbon dioxide plus the desired concentration of hydrogen cyanide.

Lowering the temperature after the steady state had been attained was found always to result in an increased fluorescence intensity. Both for very low and for very high intensities of the exciting light, however, the magnitude of the effect is small. But at intermediate light intensities sudden cooling produces a large outburst of fluorescence, reaching even twice the intensity obtained at 23°C. The fluorescence reaches a maximum and then falls again, finally becoming fairly constant after 10 or 20 min.

The final value obtained still is greater than the fluorescence intensity at room temperature, and the amount of this increase is greater than that obtained with weaker or with stronger illumination. Figure 19 and table 4 show these relations.

The curve in figure 19a shows how small an influence is produced by lowering the temperature when the light intensity is high. The curve in figure 19b demonstrates the large rise produced by decreasing the temperature at a more moderate light intensity. In this particular curve, instead of waiting until the new steady state had been attained, the temperature has been restored to 23°C. after a few minutes, and correspondingly the fluorescence returns to its former value. In the curve of figure 19c are shown the different effects produced by cooling when a given leaf is illuminated with a moderate light intensity and with a very low intensity. The points *A* and *B* represent the steady-state fluorescence for moderate and low light intensities, respectively, at room temperature. At *C* the moderate light intensity was restored and the temperature simultaneously lowered. An outburst in fluorescence intensity occurs under these conditions, but a fairly constant value has been reached after 15 min. at the point *E*, which has been taken as the steady-state value at the lower temperature. As is evident from the figure, the intensity at *E* is definitely higher than at *A*, the room temperature value for the same illumination.

The fluorescence intensities at the points of weak illumination, *B* and *F*, are very nearly equal, however.

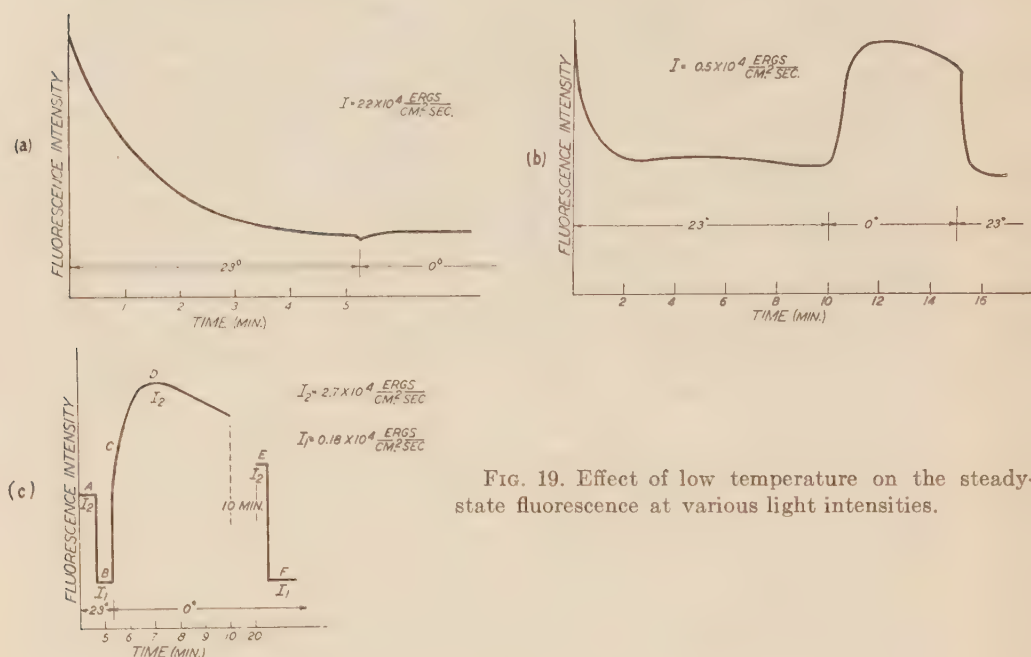


FIG. 19. Effect of low temperature on the steady-state fluorescence at various light intensities.

TABLE 4

Increase of fluorescence on cooling to $0^\circ\text{C}.$, when leaves are illuminated with light of various intensities

INTENSITY OF EXCITING LIGHT	CONSTANT LEVEL OF FLUORESCENCE AT $23^\circ\text{C}.$	NEW CONSTANT LEVEL ATTAINED AFTER SUDDEN COOLING TO $0^\circ\text{C}.$	PER CENT INCREASE IN FLUORESCENCE EFFICIENCY DUE TO COOLING AT $0^\circ\text{C}.$
<i>ergs per cm.² per second</i>			<i>per cent</i>
22×10^4	24.5	25.5	4
10×10^4	51.0	52.0	2
7×10^4	38.1	41.5	9
2.7×10^4	53.5	65.0	21
1.8×10^3	18.8	19.0	~ 1

Sample experimental curves from which this table was prepared are shown in figure 19.

It is obvious, therefore, that the same rise in fluorescence yield occurs at $0^\circ\text{C}.$ as is obtained at room temperature, except that it occurs at a much lower light intensity when photosynthesis has been inhibited by a low temperature. These relations are shown in figure 20, obtained from the data of table 4.

Experiments analogous to those carried out at room temperature (*cf.* page 1277) were performed to measure the lifetime of the highly fluorescing substances at low temperatures. A leaf was illuminated with a very high light intensity for periods of 5 and 10 min. at 0°C. Then the intensity of the exciting light was suddenly decreased. A survival of the highly fluorescing substance after reduction of the light intensity was observed, as shown in figure 21. Because of the two opposing tendencies observed on

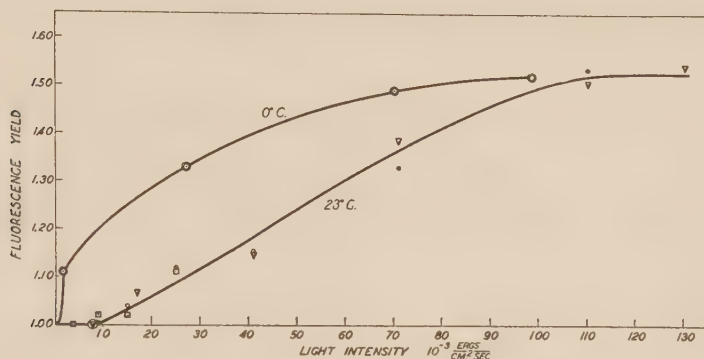


FIG. 20. Fluorescence yield as a function of the intensity of the exciting light at room temperature, and at 0°C., where photosynthesis is inhibited.

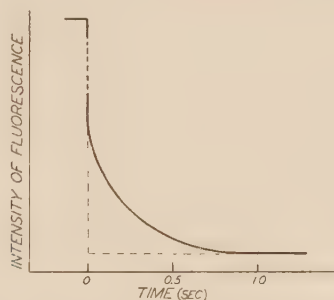


FIG. 21. Decrease of the light intensity at 0°C. is accompanied by a measurable survival of the highly fluorescing material.

page 1284, however, one must be cautious in the interpretation to be given to this result.

(b) *Inhibition by hydrogen cyanide:* The introduction of gaseous hydrogen cyanide to a photosynthesizing leaf in its steady state produces effects qualitatively the same as those due to lowering the temperature. Wassink, Vermeulen, Reman, and Katz (20) have found that in the presence of cyanide the fluorescence yield remains unaffected for small values of the exciting light, but rises when the exciting illumination is increased. As our low-temperature experiments led us to expect, we found that, if one

continues to increase the exciting light, a point is reached where the addition of hydrogen cyanide has no effect on the steady-state fluorescence of a leaf. Our results are summarized in table 5. The procedure used consisted in illuminating a leaf in an atmosphere of 1 per cent of carbon dioxide in air until a steady state had been reached. Then a new gas mixture consisting of air containing 1 per cent of carbon dioxide plus 2 per cent of hydrogen cyanide was admitted to the chamber. This concentration of hydrogen cyanide was shown by measurements with a Warburg manometer to suppress photosynthesis almost completely.

TABLE 5

Effect of 2 per cent gaseous hydrogen cyanide on the fluorescence of a leaf at various light intensities

I_E = INTENSITY OF THE EXCITING LIGHT	FLUORESCENCE IN STEADY STATE BEFORE INTRODUCTION OF HCN (ARBITRARY UNITS)	NEW CONSTANT LEVEL REACHED AFTER INTRODUCTION OF HCN	PER CENT INCREASE IN FLUORESCENCE EFFICIENCY DUE TO INTRODUCTION OF HCN
<i>ergs per cm.² per second</i>			<i>per cent</i>
0.20×10^4	51.6	58.0	12
0.47×10^4	50.5	53.8	6
7.0×10^4	32.9	32.9	0

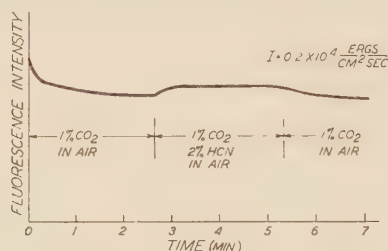


FIG. 22. Reversible increase in fluorescence intensity accompanying the addition of hydrogen cyanide to a photosynthesizing leaf in the steady state.

A sample curve is reproduced in figure 22, which also shows the restoration of the original fluorescence intensity when the leaf chamber is swept with a gas mixture free from hydrogen cyanide.

(c) *Inhibition by excess carbon dioxide:* A series of experiments was performed to determine the effect of changes in the carbon dioxide concentration during the steady state. Leaves were illuminated in a given atmosphere for 5 or 10 min., and then the concentration of carbon dioxide in the gas mixture fed to the leaf was changed. An increase in the concentration of carbon dioxide was found to have quite different effects on the fluorescence curve, depending on whether or not its initial concentration was limiting at the light intensity used.

If the concentration of carbon dioxide is initially too low, addition of a

moderate but not excessive amount of this gas produces first a dip in the fluorescence curve, then a rise, and finally a slow fall in fluorescence (figure 23). If one starts with an already sufficient supply of carbon dioxide (1 per cent or 4 per cent), however, and increases its concentration to 20 or 25 per cent, the first dip is absent,—the fluorescence intensity rises very sharply and then falls again (figure 24). The sharp fluorescence rise and slow fall shown in figures 23 and 24 are very much like the fluorescence

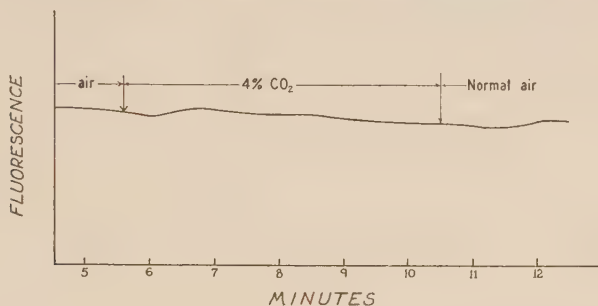


FIG. 23. Effect on the steady-state fluorescence of increasing the carbon dioxide concentration from a very low value (air) to a concentration sufficiently high so as not to limit the rate of photosynthesis (4 per cent carbon dioxide). Light intensity = 7.1×10^4 ergs per cm^2 per second.

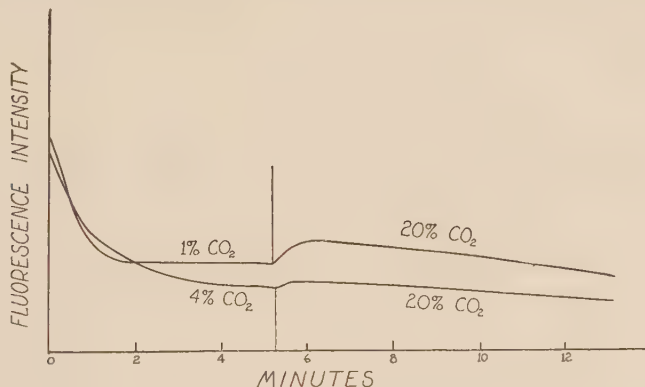


FIG. 24. Effect of increasing the carbon dioxide concentration from an initially adequate supply to an excessive amount.

behavior during the induction period. The rise which occurs on the addition of carbon dioxide is somewhat slower, doubtless because of the time necessary for the gas exchange.

As might be expected, if the change in carbon dioxide concentration is from an initially limiting value to an excessive one, both effects occur in the same curve, i. e., an initial dip in the fluorescence intensity occurs, followed by a rise, as shown in the first half of figure 25.

It is difficult to compare the final value of the fluorescence intensity in 20 per cent carbon dioxide with that in a more moderate concentration, because the fluorescence outburst vanishes very slowly in the presence of an excess of carbon dioxide and, also, the steady state itself is not quite constant at the high light intensities (compare page 1282).

Fluorescence changes similar to those just described are obtained also when the concentration of carbon dioxide surrounding the leaf is decreased from excessive values to normal ones. The last half of figure 25 presents the effects obtained when air is made to replace 25 per cent carbon dioxide in the leaf chamber.

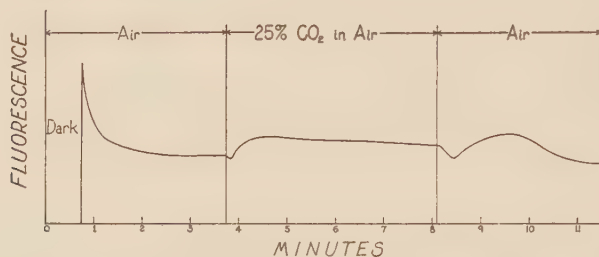


FIG. 25. Effects of increasing the carbon dioxide concentration from an initially limiting value (air) to an excessive amount (25 per cent carbon dioxide). The effect of the reverse change is also shown.

IV. DISCUSSION

A. The steady state

In our theoretical discussion, we shall first review the experimental results on the steady state. Briefly recapitulated, the main facts are these. The fluorescence yield is constant at low light intensities, but at higher illuminations it increases with the light intensity. The curve obtained is S-shaped, with the fluorescence yield approaching a value about 70 per cent greater than that obtained at low light intensities. In hydrangea leaves and in the presence of sufficient carbon dioxide, this increase in fluorescence yield occurs in the region where photosynthesis undergoes the transition to light saturation (see figure 17); in young wheat plants, although an intensity region large enough to produce saturation in the presence of an adequate supply of carbon dioxide was not experimentally accessible, a rising fluorescence yield was observed when light saturation of photosynthesis was produced by using a limiting concentration of carbon dioxide (see McAlister and Myers (9)); finally, in *Chlorocella* under the experimental conditions used by Wassink, Vermeulen, Reman, and Katz (20), very little increase in fluorescence yield was observable even at saturation light intensity.

Inhibition of photosynthesis by cyanide or low temperatures causes the

rise in fluorescence yield to occur at much lower light intensities. Both at very low and at very high light intensities, therefore, the fluorescence intensity of inhibited and uninhibited leaves is the same. At intermediate light intensities, however, the fluorescence of inhibited leaves is much greater (see pages 1287, 1288).

All these facts can be explained by assuming that these changes in fluorescence intensity are due to changes in the concentration of the molecules in contact with the chlorophyll which are capable of undergoing a photochemical reaction. This group of substances includes the compound "CO₂-acceptor molecule" and all the intermediate products of photosynthesis (see Franck and Herzfeld (4)). Substances which are incapable of taking up the excitation energy of chlorophyll and so quenching its fluorescence are the free acceptor molecules unconnected with carbon dioxide and surface-active narcotics like phenylurethan. Accumulation of any of these substances around the chlorophyll increases its fluorescence yield.

According to the theory of Franck and Herzfeld (4), at high light intensities but with other conditions normal, light saturation of photosynthesis occurs because the velocity of a dark reaction involving a catalyst called B becomes limiting. Catalyst B converts the freshly formed unstable photoproducts into a stable form capable of undergoing further photochemical reaction. The fact that the catalyst B is limiting cannot cause an increase in the fluorescence yield, because the instability of the freshly formed photoproducts prevents their accumulation about the chlorophyll. In order for the fluorescence yield to be increased, the velocity of another catalytic reaction—involving a stable substrate—must also become smaller than the velocity of the photochemical steps. This reaction, because of reasons to be discussed later, we conclude to be the carboxylation reaction in which carbon dioxide becomes joined to the acceptor molecule with the help of a catalyst called catalyst A. Whenever this reaction is depressed, as when the concentration of carbon dioxide is limiting or in the presence of specific inhibitors, the velocity of this carboxylation reaction will be too small to keep the chlorophyll supplied with photosynthetic intermediates. The concentration of carbon dioxide-free acceptor molecules in contact with the chlorophyll increases and, correspondingly, the fluorescence yield rises. A similar situation occurs at very high light intensities where, through the action of excessive back-reactions, the carboxylation reaction is reversed so that the equilibrium will be shifted in the direction of free acceptor molecules.

Under normal conditions, therefore, the light intensity at which the rise in fluorescence occurs will depend on the maximum rate of the carboxylation reaction, determined by the concentration of catalyst A molecules. By this means we are able to account for the fact that Wassink,

Vermeulen, Reman, and Katz (20) failed to obtain a rise in fluorescence of *Chlorella* even when well into the saturation region. The conclusion is that, under the conditions of those experiments, the ratio of catalyst A to catalyst B molecules was greater than the value usually obtained in hydrangea leaves.

The early rise of the fluorescence yield when photosynthesis is inhibited by cyanide or low temperatures is explained in a similar manner. Cyanide inhibits the catalytic carboxylation reaction, so that the chlorophyll becomes denuded of intermediates at moderately low light intensities and the fluorescence intensity rises.⁷ At low temperatures also, the velocity of this carboxylation reaction becomes sufficiently slow so that the same effect is produced.

On the basis of these considerations, Franck and Herzfeld (4) calculated the curve showing the change in fluorescence yield with light intensity in a normal leaf. The results are in excellent agreement with experiment (figure 17). The solid curve of figure 17 is the calculated one; the measured values are marked by circles.

B. The induction period

We can now discuss the changes in fluorescence which occur whenever the rate of photosynthesis is suddenly increased and their relation to the corresponding anomalies which are observed in the rate of photosynthesis. Two types of fluorescence curves have been observed. The type generally found in higher plants and also in algae under special culture conditions is represented by figure 2. The corresponding photosynthesis curves have been measured by Blinks and Skow, who followed the rate of oxygen production, and by McAlister and Myers, who measured the rate of carbon dioxide uptake. As McAlister and Myers have pointed out, there exists a direct antiparallelism between the rate curves of photosynthesis and the fluorescence intensity in these plants.

The second type of fluorescence curve is observed in algae cultivated under other conditions and is shown in figure 15. In these curves, the total variation in fluorescence intensity is much smaller, but the same antiparallelism is observed between the fluorescence during the induction period and the carbon dioxide uptake. The measurements of the rate of carbon dioxide uptake under these conditions have been made by Aufdemgarten (1), but corresponding studies of the oxygen production have never been performed. We have reason to believe that in this case a direct antiparallelism will not be observed.

The occurrence of a period of increasing photosynthesis and decreasing fluorescence intensity is due to the fact that, during the preceding dark

⁷ That cyanide poisons the reaction responsible for the carbon dioxide uptake was shown by Kamen *et al.* (12-17).

period, some catalyst of photosynthesis has become inactivated. The induction period of photosynthesis represents, therefore, the time necessary for the restoration of this catalyst to its normal working state. This hypothesis was first proposed by Gaffron (6). It will be used by us and extended to account for the newer experiments. The evidence for this hypothesis is as follows: The induction period cannot be due to the necessity for adjusting the concentrations of intermediates from the distribution which obtains in the dark to that established in the illuminated steady state. This conclusion is based on the following reasons: (1) Darkening the plant for a period of 1 min. is sufficient to cause a fully developed induction period to occur on re-illumination. Calculations based on the stability of the intermediates show that this time is much too short to change the distribution of the intermediates appreciably. (2) The duration of the induction period of the type shown in figure 2 is independent of the light intensity, whereas by this hypothesis one should expect the length of the induction period to be inversely proportional to the rate of photosynthesis. (3) An induction period occurs when the light intensity is suddenly increased from a low value to a higher one, despite the fact that both illuminations are still in the range where photosynthesis is proportional to the light intensity. But the concentration of photosynthetic intermediates should remain constant throughout this range, so that the induction period cannot be due to a redistribution of the concentrations of these products. Moreover, that the induction process is connected with a dark reaction is conclusively shown by the fact that in intermittent light the induction period decreases progressively the more rapidly the illumination is interrupted by dark periods (8).

The catalyst the inactivation of which in the dark is responsible for the induction period of photosynthesis is neither the catalyst A nor the catalyst B referred to previously. This conclusion was arrived at from the following considerations: The experiments involving the uptake of carbon dioxide in the dark and those of Kamen and Ruben on the exchange of normal and radioactive carbon dioxide show that the total concentration of intermediates present is comparable to that of the chlorophyll. If the induction period were due to a limitation in the action of catalyst A, on illumination one should observe an immediate influence on the carbon dioxide uptake only, but neither the fluorescence intensity nor the oxygen evolution should be affected at the very beginning. This, however, is not the case, at least in the normal type of induction curves.

On the other hand, an inactivation of catalyst B would affect the oxygen production and the carbon dioxide uptake, but not the fluorescence intensity at all, as discussed in the section on steady fluorescence.

Therefore the catalyst involved must be a third one, C. The liberation of oxygen from the peroxides in photosynthesis has always been assumed

to proceed through the action of a catalyst. It is natural, therefore, tentatively to identify C with this catalyst.

It is necessary then to find a process which inactivates catalyst C in the dark and re-activates it in the light. In all probability the deactivation is a slow oxidation process connected with the plant metabolism. In the light this process would be counterbalanced by a slow reduction process for which the photochemical end product of photosynthesis may be responsible. For, although the over-all reaction of photosynthesis must produce oxidizing and reducing substances in equal amounts, the concentration of the reducing agents will be greater inside the chloroplasts because of their longer lifetime, i.e., in the steady state of photosynthesis the peroxides (oxidizing agents) will have a very short lifetime, since they are quickly decomposed by catalyst C and the molecular oxygen produced escapes. The photochemical end products, which are reducing agents, may diffuse to other parts of the cell or undergo chemical transformations, but these processes are certainly slower than the time of removal of the peroxide. Therefore, the concentration of reducing substances in the chloroplast will increase directly with photosynthesis, as will the fraction of catalyst C which is in an active state. The fact that the concentration of active catalyst C varies directly with the rate of photosynthesis readily explains the occurrence of a new induction period whenever the rate of photosynthesis is suddenly increased. The concentration of active catalyst C is temporarily insufficient to handle all the peroxide molecules produced.

According to this picture, the period of darkness necessary for the development of a new induction period on re-illumination (about 1 min. in leaves) represents the time necessary either for the removal of the reducing substances (by diffusion or other processes) which maintain catalyst C in an active state, or for the oxidation of catalyst C after their removal.

The variation of the fluorescence intensity with time predicted by this theory is as follows: At room temperature a plant which has had a dark period of several minutes in the presence of an adequate amount of carbon dioxide has practically the same distribution of the intermediates of photosynthesis as it had at the end of the preceding illumination period. On the instant of illumination, therefore, the photochemical steps in the photosynthetic process immediately proceed at their normal rate. The step involving liberation of oxygen from the peroxides acts as a bottleneck, however, because of the partial inactivation of catalyst C, so that peroxides will start to accumulate. The peroxide concentration thus tends to increase linearly with the time of irradiation, until so much peroxide is formed that it starts to crowd out the other intermediates in contact with the chlorophyll. When the excess peroxide begins competing seriously

with the other intermediates for the positions about the chlorophyll, the photochemical steps of photosynthesis are unable to proceed as rapidly and the rate of peroxide production decreases. Simultaneously with these processes more and more of catalyst C is being activated by the products of photosynthesis, so that the rate of removal of peroxides is being increased. The concentration of peroxides therefore at first rises, attains a maximum, and then falls, finally reaching a low constant level during steady photosynthesis.

Since the accumulation of the peroxides represents a replacement of the photosensitive substances at the chlorophyll by insensitive ones, it will be attended by a rise in fluorescence intensity. On illumination, therefore, the fluorescence intensity will start at a normal level and then rise at a rate proportional to that of photosynthesis. In less than a minute the accumulation of peroxides slows down, both because of its own effect in depressing photosynthesis and because of the increasing concentration of catalyst C. The fluorescence rise, therefore, is checked. When enough catalyst has been activated so that it not only can keep up with the instantaneous peroxide production but also slowly remove that which has already accumulated, the fluorescence curve will start down.

One can similarly account for the time course of the carbon dioxide uptake. The rate of carbon dioxide absorption should start at the normal level but then drop at the same time that the fluorescence is rising (because the competition of the peroxide molecules for the positions at the chlorophyll will decrease photosynthesis). After reaching a minimum which coincides in time with the maximum of fluorescence, the rate of carbon dioxide uptake will rise slowly to its original level. The rate of oxygen production should follow a somewhat different course for the first few minutes. It should start low and rise continuously until the final rate is reached, because the concentration of catalyst B, which controls the rate of oxygen liberation, continuously increases.

The predictions of this theory agree very well with the actual rate of change in fluorescence intensity and carbon dioxide uptake for algae of the type for which the fluorescence curve is shown in figure 15, and the photosynthesis of which was studied by Aufdemgarten. The only difference between the theory and experiment is that the fluorescence curves actually observed display a sharp initial peak in fluorescence intensity at the very beginning, which is not predicted by the theory as presented so far (for an explanation of this phenomenon, see page 1298).

We can also explain the time course of photosynthesis and fluorescence observed in higher plants and in algae cultivated under somewhat different conditions (figure 2) by postulating that some metabolic product present in larger concentration in these plants than in the others enters into the course of the reactions. This hypothesis seems plausible in view of the

fact that the algae showing fluorescence curves like that of figure 15 are poorly nourished (McAlister and Myers (9)), or have a very low respiration (Wassink *et al.* (18-20)), or are from an old culture (experiments of this paper). In all these cases the concentration of some metabolic factor might easily be reduced. Such a substance, when present in sufficiently high concentration in the chloroplasts, will be partially oxidized by the peroxides which accumulate at the beginning of an illumination.

The fluorescence curve obtained under these conditions (figure 2) has but a single maximum which is attained in about 1 sec. The highest fluorescence attained is about 300 per cent greater than the initial intensity, instead of about 20 per cent as the second maximum in figure 15; here, too, however, the fall is slow, lasting about 2 min. The rate of change of the carbon dioxide uptake is always in the opposite direction to that of the fluorescence intensity.

Our picture of the course of events when illumination begins is as follows: As before, peroxides accumulate because the amount of active catalyst C is too small. After a few tenths of a second, however, the concentration of peroxides becomes great enough to oxidize the substance X (of metabolic origin) to the product we shall call Y. This material must be able to cause the formation of the maximum in fluorescence intensity shown in figure 2 and the minima in carbon dioxide uptake and oxygen production which occur after the first second of illumination, as found by McAlister and Myers and by Blinks and Skow.

The concentration of Y is not likely to exceed the amount of peroxide produced in about 1 sec. of photosynthesis. One can show by a calculation that its concentration must be 1/50th to 1/100th that of the chlorophyll. So small an amount of material could not easily depress photosynthesis and increase the fluorescence intensity to the extent actually observed if it were to act only as a narcotic substance which covers the whole surface of the photosynthetic apparatus,—chlorophyll and catalysts alike. One must suppose, therefore, that Y is preferentially adsorbed on the catalysts.⁸ In order that an increase in fluorescence yield of the proper magnitude be observed, it is necessary, if only 2 per cent of the chlorophyll molecules are to be affected, that the fluorescence efficiency of the chlorophyll in this condition be about 100 times greater than that of the rest. This fluorescence efficiency is exhibited by chlorophyll in inert organic solutions. But whether the increase in fluorescence yield is due only to the protective action of an inert layer, or to some interaction of Y with the protein material to which the chlorophyll is attached, it is premature to decide.

At the beginning of illumination, therefore, Y is formed after a very short accumulation of peroxides (a few tenths of a second). The formation of Y quickly depresses photosynthesis by poisoning catalyst B. The carbon

⁸ We are sure only that catalyst B is poisoned by Y. It is impossible as yet to determine, nor is it important for the present purpose, whether C is also depressed.

dioxide consumption and oxygen production drop, and the fluorescence intensity shoots up. The resulting rate of photosynthesis is so low that catalyst C is no longer limiting, but is able to keep up with the new rate of peroxide production. The rate of formation of Y therefore decreases considerably.

The subsequent decline of fluorescence intensity and slow recovery of photosynthetic activity is caused by the fact that the removal of Y occurs at a rate exceeding its formation. The experiments of page 1277 show that Y disappears in a dark reaction and that it has a lifetime of a few seconds. Probably it is consumed by respiration or a similar metabolic process (support for this assumption will be presented below). The production of reducing substances through the agency of the small amount of catalyst C which is in an active state in turn causes more of the active catalyst C to become available. The removal of peroxides is therefore hastened and the formation of Y is reduced. Since Y is continuously being removed by a dark reaction while its formation gradually diminishes, the rate of photosynthesis slowly increases. In spite of their increased production, peroxides are now easily handled by the greater supply of catalyst C available. In this manner the level of Y falls, photosynthesis builds up to its steady-state value, and the fluorescence intensity returns to normal.

The whole mechanism therefore functions so that the plant is protected against the effects of sudden, excessive illumination. If more light is admitted to it than the photosynthetic mechanism is prepared to handle, the photochemical reactions are automatically inhibited until sufficient catalyst has been made available by the plant to remove the peroxides as fast as they are formed. In this way accumulation of substances which might be injurious to the plant is prevented.

This picture of the induction period of photosynthesis enables the other observations presented in this paper to be readily understood. That a fluorescence outburst occurs when the rate of photosynthesis is increased not only by increasing the light intensity, but also when carbon dioxide is added to a leaf previously illuminated in a carbon dioxide-limiting atmosphere, is self-evident. Similarly, the prolongation of the induction period by the application of low temperature, by an excess of carbon dioxide, and, to some extent, by hydrogen cyanide can be readily understood.

In the case of low temperature the lifetime of Y is increased, as our experiments have shown; in the presence of an excess of carbon dioxide, the metabolism is known to be greatly changed. Both the rate of formation and the rate of removal may be affected so that the level of Y becomes temporarily increased. The effect of hydrogen cyanide is similar, but smaller in magnitude. The addition of any of these agents after the steady state of photosynthesis has been achieved also produces a fluorescence outburst by the same mechanism.

Next we may discuss the relationship between the fluorescence outburst

and the light intensity. As discussed on page 1272, the time necessary for the initial fluorescence rise is inversely proportional to the rate of photosynthesis. This is to be expected, since the fluorescence outburst is directly coupled to the peroxide production. The time necessary for the fluorescence decay, however, is independent of the light intensity. An explanation of this emerges from the following consideration: At the time of the fluorescence maximum, the amount of active catalyst C available is the same regardless of the light intensity. (This amount may be the same as that which obtains in the dark.) The rate of activation of C is proportional to that of photosynthesis so that, even though more of the active form is required at the higher light intensity, its rate of production is also greater. Thus the time necessary to achieve the steady state is practically constant, at moderately low or at high light intensities. At very low light intensities, the whole outburst becomes less pronounced, because the rate of photosynthesis is so low that the level of active catalyst present in the dark then becomes significant.

It is also possible to explain the phenomenon described on page 1284 wherein a large decrease in the intensity of the exciting light is followed by a gradual rise in fluorescence intensity. It has already been shown that a dark period of about 1 min. is necessary in order for a complete fluorescence outburst to appear on re-illumination. This is to be interpreted to mean that it requires about 1 min. to inactivate the catalyst C to the level which obtains in the dark. A similar process should take place when the light intensity is decreased to a very small value. The sudden transition from strong to weak light will leave a temporary surplus of catalyst C, the inactivation of which will take about 1 min. The concentration of the small amount of Y always present in the steady state will therefore be even smaller than normal, but will increase throughout this period to its steady-state value. Thus one observes a gradually increasing fluorescence for about 1 min.

The explanation here presented for the two different kinds of induction periods and fluorescence-time curves observed in plants is based on the assumption that in certain plants only a substance X is present in sufficient quantity to affect appreciably the course of the reactions of photosynthesis. It is hardly likely that this metabolic substance, X, would be completely absent in plants cultivated under somewhat different conditions. That some X is present in those plants also is seen from the following considerations: In the discussion of figure 15, no explanation was given for the initial sharp peak in fluorescence intensity during the first few seconds of illumination. The size of this fluorescence peak depends very markedly on the state of metabolism of the plant, since, as Wassink and Katz (19) have shown, reduction of the oxygen pressure below the limit necessary for normal respiration causes a great increase in the intensity of this fluorescence maximum. It seems probable, there-

fore, that these plants contain a small amount of X. This material would undergo the same fate that it undergoes in the other plants but in a much shorter time, since there is so little material present. Thus, one obtains a very short-lived fluorescence rise and fall.

Finally, we have to discuss the effect on the time course of fluorescence which is due to an anomalous distribution of intermediates in the photosynthetic system. The curves like that of figure 8 belong to this group. These fluorescence curves were obtained when leaves, cultivated in normal air where carbon dioxide is limiting, were placed in an atmosphere containing a sufficient supply of carbon dioxide. The plant contained, therefore, a deficient amount of the photosynthetic intermediates. Addition of carbon dioxide in the dark now causes the free acceptor molecules to absorb this gas and form a reservoir of RCOOH which is larger than usual. When the plant is first illuminated, the fluorescence outburst will depend only on the concentration of the higher intermediates, and so will look the same as a curve made in air. After some time, however, the excess of the RCOOH will have been promoted to the peroxide stage. Since catalyst C is not yet active enough to handle this excess, another fluorescence outburst occurs.

Related to these phenomena are the observations made by McAlister, wherein a rise of fluorescence coincided with an increasing rate of carbon dioxide uptake. These measurements were made under conditions of severe limitation of carbon dioxide. Again, during the dark period preceding illumination, an amount of RCOOH will be produced which is large compared to the concentrations of the other intermediates. On again irradiating, the time course of fluorescence starts out as usual. But the effect of the slow depletion of the RCOOH from the photosynthetic apparatus will overlap on the curve observed, producing a slow fluorescence rise. Since the plant has started with a surplus of RCOOH at the beginning of illumination, it will not take up as much carbon dioxide as it will later in the steady state.

Very similar considerations explain the dependence of the time course of fluorescence on the concentration of cyanide in the observations of Wassink and Katz (19). The poisoning of the carboxylation reaction is responsible for the denudation of intermediates about the chlorophyll in the steady state of irradiation. During a dark period, a surplus of RCOOH will again be formed, and again a rising fluorescence will be superimposed on the normal curve.

V. SUMMARY

1. The fluorescence anomalies of the chlorophyll in leaves and algae during the induction period of photosynthesis have been observed under various conditions of temperature, light intensity, and gas concentration, etc. Curves showing the fluorescence intensity as a function of time of

irradiation have been measured and recorded photographically by means of a photocell amplifier system.

2. The intensity of the steady-state fluorescence which is attained after an irradiation of several minutes has been measured as a function of the intensity of the exciting light. Above a certain minimum intensity, the fluorescence yield increases with the strength of the exciting illumination. The curve obtained fits the calculations made by Franck and Herzfeld.

3. The effect of photosynthetic inhibitors on the steady-state fluorescence has been studied.

4. A theory has been presented which explains the induction period of photosynthesis and the related fluorescence anomalies.

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REFERENCES

- (1) AUFDEMGARTEN, H.: *Planta* **29**, 643 (1939); **30**, 343 (1939).
- (2) BLINKS, L. R., AND SKOW, R. K.: *Proc. Natl. Acad. Sci. U. S.* **24**, 420 (1938).
- (3) FRANCK, J., AND FRENCH, C. S.: Unpublished results, to appear in the *Journal of General Physiology*.
- (3a) FRANCK, J., AND GAFFRON, H.: *Advances in Enzymology*, Vol. I. Interscience Publishers, New York (1941).
- (4) FRANCK, J., AND HERZFELD, K. F.: *J. Phys. Chem.* **45**, 978 (1941).
- (5) FRANCK, J., AND WOOD, R. W.: *J. Chem. Phys.* **4**, 551 (1936).
- (6) GAFFRON, H.: *Naturwissenschaften* **25**, 460, 715 (1937).
- (7) KAUTSKY, H., AND COWORKERS: *Naturwissenschaften* **19**, 964 (1931); **19**, 1043 (1931); **24**, 317 (1936); **26**, 576 (1938); *Ber.* **65**, 1762 (1932); **66**, 1588 (1933); **68**, 152 (1935); *Biochem. Z.* **274**, 423 (1934); **274**, 435 (1934); **277**, 250 (1935); **278**, 373 (1935); **302**, 1137 (1939).
- (8) McALISTER, E. D.: *Smithsonian Inst. Pub., Misc. Collections* **95**, No. 24 (1937).
- (9) McALISTER, E. D., AND MYERS, J. E.: *Smithsonian Inst. Pub., Misc. Collections* **99**, No. 6 (1940).
- (10) MYERS, J. E., AND BURR, G. O.: *J. Gen. Physiol.* **24**, 45 (1940).
- (11) ORNSTEIN, L. S., WASSINK, E. C., REMAN, G. H., AND VERMEULEN, D.: *Enzymologia* **5**, 110 (1938).
- (12) RUBEN, S., HASSID, W. Z., AND KAMEN, M. D.: *J. Am. Chem. Soc.* **61**, 661 (1939).
- (13) RUBEN, S., AND KAMEN, M. D.: *Proc. Natl. Acad. Sci. U. S.* **26**, 418 (1940).
- (14) RUBEN, S., AND KAMEN, M. D.: *J. Am. Chem. Soc.* **62**, 3451 (1940).
- (15) RUBEN, S., KAMEN, M. D., AND HASSID, W. Z.: *J. Am. Chem. Soc.* **62**, 3443 (1940).
- (16) RUBEN, S., KAMEN, M. D., HASSID, W. Z., AND DEVAULT, D. C.: *Science* **90**, 570 (1939).
- (17) RUBEN, S., KAMEN, M. D., AND PERRY, L. H.: *J. Am. Chem. Soc.* **62**, 3450 (1940).
- (18) VERMEULEN, D., WASSINK, E. C., AND REMAN, G. H.: *Enzymologia* **4**, 254 (1937).
- (19) WASSINK, E. C., AND KATZ, E.: *Enzymologia* **6**, 145 (1939).
- (20) WASSINK, E. C., VERMEULEN, D., REMAN, G. H., AND KATZ, E.: *Enzymologia* **5**, 100 (1938).

